REMARKS/ARGUMENTS

Claims 1-3, 6-12, 14-15, 17, 59, and 66-89 are pending. Claims 4-5, 13, 16, 18-19, and 60-65 are cancelled.

New claims 66-79 and 80-89 are similar to claims 1-3, 6-12, 14-15, 17, 59, except that the host cell is yeast.

Support for amended claims 2, 3, 6, and new claims 66-69 and 80-81 can be found in the specification in paragraphs [0145]-[0153] and in Example 2.

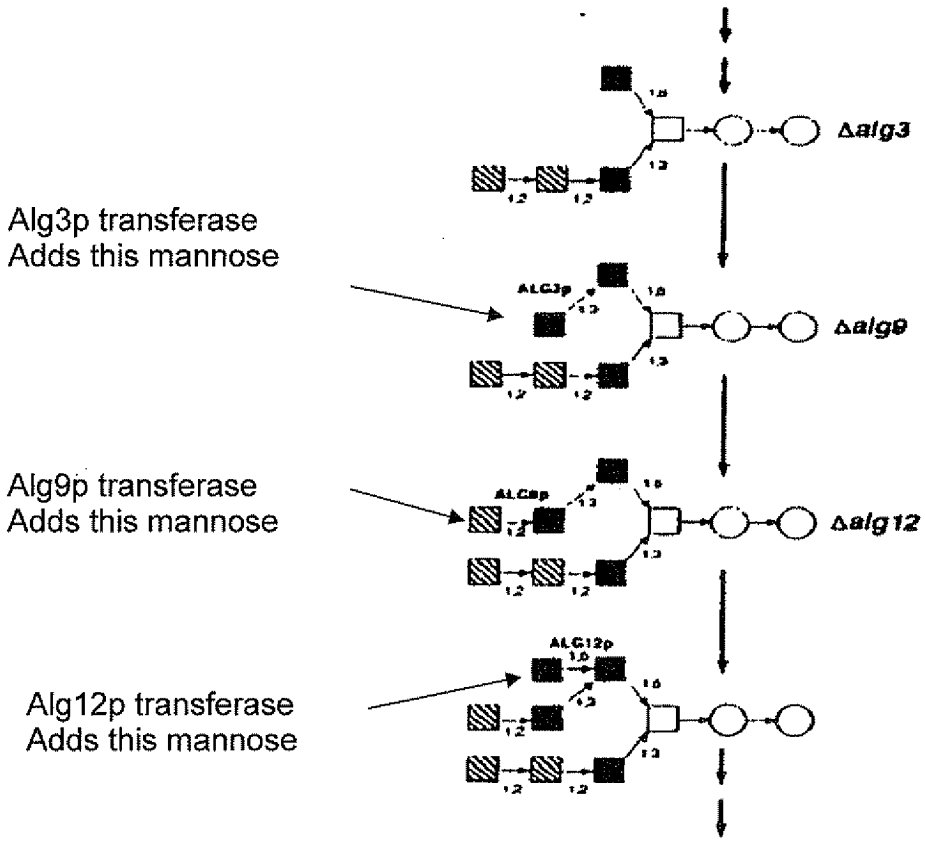
Support for the sugar transporters in claims 70 and 82 can be found in paragraphs [0159]-[0162].

Support for the various transferases in amended claim 7 and new claims 71 and 80 can be found in paragraph [0153], lines 8-15, and original claims 32-34.

I. Claim rejections 35 U.S.C. § 112

Claims 1-3, 6-12, 14-15, and 59 are newly rejected under 35 U.S.C. § 112, first paragraph. The rejection states that "[w]hile the specification and the art provides adequate written description for Alg12 being an enzyme that transfers a sugar residue to the 1,6 arm of a lipid-linked oligosaccharide structure, the specification fails to adequately describe other enzymes that have this biological activity . . . Therefore, only Alg12 meets the written description provision of 35 U.S.C. §112, first paragraph."

The applicants respectfully disagree. Figures 1 and 2 show that the Alg3p and Alg9p enzymes also transfer a mannose residue to the 1,6 arm of the lipid-linked oligosaccharide. The relevant part of Figure 2 has been reproduced below and annotated to show the activity of the three enzymes that add sugars to the 1,6 arm of lipid linked oligosaccharides.



As shown, Alg3p attaches the mannose to the 1,6 arm in an alpha-1,3 linkage. The term "alpha-1,3-mannosyltransferase" in the name of the enzyme refers to the sugar linkage catalyzed by the enzyme and not to the arm of the lipid-linked oligosaccharide it puts the sugar on. The term "$Man_5GlcNAc_2$" in the name refers to the lipid-linked substrate the Alg3p acts on. The "$Man_5GlcNAc_2$" substrate is shown in Figure 2 at the top left. The product of the Alg3p activity is a lipid-linked oligosaccharide that has six mannose residues.

Alg9p (dolichyl-P-Man:$Man_6GlcNAc_2$-PP-dolichyl alpha-1,2 mannosyltransferase) is next in the pathway and it attaches a mannose to the 1,6 arm of the lipid-linked oligosaccharide in an alpha-1,2 linkage. The product of the Alg9p activity is a lipid-linked oligosaccharide that has seven mannose residues.

Alg12p 9 dolichyl-P-Man:$Man_7GlcNAc_2$-PP-dolichyl alpha-1,6 mannosyltransferase) is next in the pathway and it attaches the mannose to the 1,6 arm of the lipid-linked oligosaccharide in an alpha-1,6 linkage.

The Examples show the construction of recombinant *Pichia pastoris* in which the *ALG3* gene has been deleted. For example, page 55, paragraph [0177], describes the

construction of a *K. lactis* strain in which the *ALG3* gene had been deleted and page 57, paragraph [0182], shows the construction of strain RPD27 in which the *ALG3* gene had been deleted. Example 3 on page 61 describes how to identify and delete the *ALG9* and *ALG12* genes in *Pichia pastoris*. Figure 2 of Burda et al., Biochimica et Biophysica Acta 1426: 239-257 (1999) cited in the 35 U.S.C. § 103(a) rejection in the instant Office Action also shows that Alg3p and Alg9p also transfer mannose residues to the mannose residues on the 1,6 arm of the lipid-linked oligosaccharide.

In light of the above, it is believed that the currently pending claims comply with the written description requirement of 35 U.S.C. § 112, first paragraph. Reconsideration of the rejection is requested.

II. Claim rejections 35 U.S.C. § 112

Claims 8 and 9 are newly rejected under 35 U.S.C. § 112, second paragraph. The rejection states that "Alg3 (dolichyl-P-Man:Man5GlcNAc2, PP-dolichyl alpha-1,3 mannosyltransferase, in claims 8 and 9, is not an enzyme that transfers a sugar residue to the 1,6 arm of an oligosaccharide. It transfers a sugar to the 1,3 arm of the sugar."

The applicants respectfully disagree. As shown in Figures 1 and 2 and explained above, the Alg3p enzyme transfers a mannose residue to the 1,6 arm of the lipid-linked oligosaccharide.

In light of the above, it is believed that claims 8 and 9 comply with 35 U.S.C. § 112, second paragraph. Reconsideration of the rejection is requested.

III. Claim rejections 35 U.S.C. § 103

Claims 1-3, 6, 7, 10-12, 14, 15, 17, and 59 have been newly rejected under 35 U.S.C. § 103(a) as being unpatentable over Goss et al., 1995, Clinical Cancer Res. 1: 935-944; Yoshida et al. WO0034490 published June 15, 2000; Gemmill et al., 1999, Biochimica et Biophysica Acta 1426: 227-237; Burda et al., 1999a, Biochimica et Biophycica Acta 1426: 239-257; Karaoglu et al., 2001, Biochemistry 40: 12193-12206; Burda et al., 1999b, Glycobiology 9: 617-625; Tremblay et al., 1998, Glycobiology 8: 585-595; Sarkar et al., 1991, PNAS USA 88: 234-238; and Moreman et al., 1991, The Journal of Cell Biology 115: 1521-1534.

The rejection alleges that all of the component parts of the instant invention are taught in the above-cited references in which '[t]he only difference is the combination of 'old elements' into a single method of producing a recombinant protein in a unicellular or filamentous fungus, wherein the recombinant protein has an N-glycans GlcNAcMan3GlcNAc2 attached to it." The rejection further states "It would have been obvious for an ordinary artisan to take

mutant yeast that synthesize oligosaccharides of a desired structure and to modify the oligosaccharide to GlcNAcMan3GlcNAc2 such that an artisan would arrive at a glycoprotein that can be modified for cancer studies. With regards to particular limitations of the claims, Burda et al., 1996b teach a yeast with a mutation in transferring sugar to the 1,6-arm of a lipid-linked oligosaccharide was known (Alg12) and that those oligosaccharides can be transferred to an asparagine residue on a protein (Karaoglu et al.). Enzymes (and their coding sequences) that remove sugars and add GlcNAc were also known (Yoshida, Tremblay et al., Sarkar et al, and Moremen et al.). Thus, the claims are obvious."

The applicants respectfully disagree with the rejection. The currently claimed invention provides yeast or filamentous fungi that have been genetically engineered to make glycoproteins that have complex mammalian or human-like *N*-glycans, not yeast or filamentous fungus *N*-glycans. In general, the cited art does not teach or suggest genetically engineering yeast or filamentous fungi to lack Alg3, Alg9, and/or Alg12 activity and to further include the mammalian enzymes necessary to enable the yeast or filamentous fungus to make glycoproteins that have complex mammalian- or human-like *N*-glycans. It is believed that the cited art as a whole does not teach or suggest all the elements necessary for a person skilled in the art to make the currently claimed invention without undue experimentation. For example, the art cited in the rejection does not teach or suggest the following elements.

The applicants teach that to make such genetically engineered organisms, the organism must lack α1,6-mannosyltransferase activity. In the examples, the yeast *Pichia pastoris* was genetically engineered to lack the α1,6-mannosyltransferase activity encoded by the *OCH1* gene. Deletion of the α1,6-mannosyltransferase activity prevents outer chain elongation of *N*-linked oligosaccharides in yeast and thus the occurrence of highly mannosylated or hypermannosylated *N*-glycans (*See* for example, specification: paragraphs [0125] and [0138] and JP 8-336387 submitted with an English translation in an Information Disclosure Statement submitted with this reply). Filamentous fungi do not appear to encode an α1,6-mannosyltransferase activity. The art cited in the rejection does not appear to teach or suggest that to produce a host cell capable of making glycoproteins with complex mammalian- or human-like *N*-glycans, the host cell needs to lack α1,6-mannosyltransferase activity.

The applicants teach that the mammalian glycosylation enzymes must be targeted to the appropriate location in the ER or Golgi apparatus and that this can be accomplished by taking the enzyme catalytic domain and fusing it to an appropriate targeting sequence that targets the catalytic domain to a location in the ER or Golgi that enables it to modify glycoproteins as they pass through. None of the cited art teaches or suggests this concept of locating the "right" enzyme to the "right" location. For example, Tremblay teaches expressing an α1,2-mannosidase

in *Pichia pastoris*, however, what was expressed was the soluble domain of the α1,2-mannosidase fused to the α-factor secretion signal. The recombinant *Pichia pastoris* host cell further included the *OCH1* gene and thus would be expected to make highly mannosylated *N*-glycans. Yoshida teaches expressing a human galactosyltransferase in plant cells for the purpose of providing a plant host cell capable of producing glycoproteins that have complex human-like *N*-glycans. However, Yoshida does not teach fusing the catalytic domain to a targeting peptide that targets the ER or Golgi. Unlike yeast and filamentous fungus cells, plant cells produce glycoproteins that have complex *N*-glycans. The object of Yoshida was to provide recombinant plant cells that make glycoproteins with galactose-terminated complex *N*-glycans. Yoshida does not appear to suggest using yeast or filamentous fungi to produce glycoproteins that have complex *N*-glycans.

The other cited art that relates to yeast cells are (1) Gemmill, which is a review of *N*- and *O*-glycosylation in yeast, but which does not address the problem solved by the applicants, i.e., to make a yeast or filamentous fungus cell that can make glycoproteins with mammalian- or human-like *N*-glycans; (2) Karouglu, which discusses the oligosaccharide transferase system in yeast that transfers the lipid-linked oligosaccharide to the Asn residue of a glycoprotein but which does not address the problem solved by the applicants, i.e., to make a yeast or filamentous fungus cell that can make glycoproteins with mammalian- or human-like *N*-glycans; (3) Burda 1999a, which is review article on the dolichol pathway but which does not address the problem solved by the applicants, i.e., to make a yeast or filamentous fungus cell that can make glycoproteins with mammalian- or human-like *N*-glycans; and (4) Burda 1999b, which teaches the *ALG12* locus in yeast, but which does not address the problem solved by the applicants, i.e., to make a yeast or filamentous fungus cell that can make glycoproteins with mammalian- or human-like *N*-glcyans. The remainder of the cited art is concerned with non-yeast or filamentous fungi cells (Sakar, Moreman, Yoshida, and Goss) and does not address the problem solved by the applicants, i.e., to make a yeast or filamentous fungus cell that can make glycoproteins with mammalian- or human-like complex *N*-glycans.

In summary, the cited art does not teach or suggest the currently claimed invention. The cited art does not appear to suggest modifying yeast or filamentous fungi to produce glycoproteins that have mammalian- or human-like complex *N*-glycans. The cited art does not provide or suggest all the tools necessary for converting yeast or filamentous fungi into organisms that can make mammalian- or human-like complex *N*-glycans. Without the teachings of the applicants' disclosure, a person of ordinary skill in the art in view of all of the cited art would not likely have arrived at the currently claimed invention without undue and non-routine experimentation. For example, the cited art does not teach or suggest the need that the host cell

lack the ability to make highly mannosylated *N*-glycans or how to render the host cells incapable of making mannosylated *N*-glycans and does not teach or suggest the need to target the glycosylation enzymes to particular locations in the ER or Golgi by fusing to a heterologous targeting peptide that targets the ER or Golgi or how to target the glycosylation enzymes to the appropriate location.

Therefore, in light of the above, the currently pending claims are believed to be patentable over the prior art. Reconsideration of the rejection is requested.

In view of the foregoing remarks and amendments, it is believed that the grounds of rejections have been overcome and that the claims are in proper condition for allowance. Accordingly, Applicants respectfully request that all of the rejections be withdrawn and a Notice of Allowance be forwarded to the Applicants. The Examiner is invited to contact Applicants' Attorney at the telephone number given below, if such would expedite the allowance of this application.

CONDITIONAL PETITION

Applicants hereby make a Conditional Petition for any relief available to correct any defect in connection with this filing, or any defect remaining in this application after this filing. The Commissioner is authorized to charge deposit account 13-2755 for the petition fee and any other fee(s) required to effect this Conditional Petition.

Respectfully submitted,

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